TECHNICAL REPORT
NATICK/TR-81/029

BIODEGRADATION OF NITRATE ESTERS USED AS MILITARY PROPELLANTS - A STATUS REPORT -

by
J. H. Cornell,
T. M. Wendt,
N. G. McCormick,
D. L. Kaplan
and
A. M. Kaplan

August 1981

UNITED STATES ARMY
NATICK RESEARCH and DEVELOPMENT LABORATORIES
NATICK, MASSACHUSETTS 01760

Science and Advanced Technology Laboratory

Citation of tradenames in this report does not constitute an official endorsement or approval of the use of such items.

Destroy this report when no longer needed.
Do not return it to the originator.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER NATICK/TR-81/029	2. GOVT ACCESSION NO. AD-A149665	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) BIODEGRADATION OF NITRATE ESTERS USED AS MILITARY PROPELLANTS - A STATUS REPORT		5. TYPE OF REPORT & PERIOD COVERED Final Report August 1981 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) J. H. Cornell, T. M. Wendt, N. G. McCormick, O. L. Kaplan and A. M. Kaplan		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Science and Advanced Technology Laboratory, DRONA-YEP, US Army Natick Research and Development Laboratories, Natick, MA 01760		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS Program Element 6.2 Project 1L1627200048 Work Unit No. 02-007
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Toxic & Hazardous Materials Agency CODE: DRXTH-TE-D Bldg. E 4585, Aberdeen Proving Ground, MD 21010		12. REPORT DATE August 1981 13. NUMBER OF PAGES 23
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release, distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

BIODEGRADATION	ESTERS	POLLUTION ABATEMENT
NITRATE ESTERS	TRIMETHYLOLETHANE	THIN LAYER CHROMATOGRAPHY
PROPELLANTS	PROPYLENE GLYCOL	GAS CHROMATOGRAPHY
POLYETHYLENE GLYCOLS	GLYCOLS	MASS SPECTROMETRY
NITRATES	MUNITION WASTES	SYNTHESIS

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The biodegradation of four glycol nitrates used as military propellants was investigated. Propylene glycol dinitrate, diethylene glycol dinitrate, triethylene glycol dinitrate and trimethylolethane trinitrate underwent sequential hydrolytic cleavage of nitrate groups under aerobic batch or continuous fermentation. Activated sludge inocula, mineral salts, and ethanol as an additional carbon source were used. The substrates and partially nitrated metabolites were monitored by thin-layer chromatography. The concentrations of the nitrate esters could be reduced below detectable limits by choice of retention time.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

20. Abstract (cont'd)

The identities of the metabolites were confirmed by gas chromatography - mass spectrometry using authentic samples whose syntheses are described. A program to obtain bench scale data for development of a microbiological treatment process for waste waters containing nitrate ester propellants is recommended.

PREFACE

Nitrate esters are employed in the formulation of propellants for military uses. Among those of current interest are propylene glycol dinitrate, diethylene glycol dinitrate, triethylene glycol dinitrate and trimethylolethane trinitrate. In connection with a study directed toward elimination of nitroglycerine from waste streams by microbial degradation, Chemical Systems Laboratory (CSL) and its successor responsible activity, U.S. Army Toxic and Hazardous Materials Agency (USATHAMA), requested Natick Laboratories to concurrently investigate the biodegradation of these four esters.

This is a status report encompassing the microbiological and chemical studies carried out pursuant to their request under Project 1L162720D048. Results obtained to date are presented and areas where final confirmatory studies would be required to develop a microbiological abatement process are indicated.

We gratefully acknowledge the aid of Mr. Carmine DiPietro of the Environmental Analysis Group who carried out the gas chromatographic/mass spectral (GC/MS) analyses in connection with these studies.

TABLE OF CONTENTS

LIST OF FIGURES

BIODEGRADATION OF NITRATE ESTERS USED AS MILITARY PROPELLANTS - A STATUS REPORT

INTRODUCTION

The four nitrate esters propylene glycol dinitrate (PGDN), diethylene glycol dinitrate (DEGDN), triethylene glycol dinitrate (TEGDN), and trimethylolethane trinitrate (TMETN) are employed in the formulation of special military propellants. They are not used in amounts comparable to other ingredients such as nitroglycerine (NG), but are prepared in batch quantities as required by the Army. Therefore waste streams from a facility such as Radford Army Ammunition Plant (RAAP), Radford, VA might contain any or all of the four esters in addition to the NG normally present. Natick Laboratories has demonstrated the basic feasibility of elimination of the NG from simulated waste waters on bench scale using an activated sludge system under aerobic conditions.[1] It was of great interest to determine whether the proposed biological treatment for NG would also be effective in eliminating the four esters without essential modification in operating conditions. The work described in this report is an initial study to determine whether significant microbiological attack occurs on the four esters of interest under conditions which were found effective for the biodegradation of NG.

The objectives of this research were: (1) to determine whether the four propellant nitro esters are biodegradable, (2) to characterize the products and intermediates resulting from the biodegradation, and (3) to synthesize and characterize those intermediates which are not otherwise available.

[1] T. M. Wendt, J. H. Cornell, and A. M. Kaplan. 1978. Microbial Degradation of Glycerol Nitrates. Appl. Env. Microbiol. 36: 693-699.

DISCUSSION

Microbial degradation of NG and its nitrate-containing metabolites has already been reported.[2] The four nitrate ester propellants were challenged by microorganisms under similar conditions, for example, batch or continuous (chemostat) culture under aerobic conditions using an activated sludge inoculum. The progress of the biotransformation and the appearance of nitrate-containing metabolites was followed by thin-layer chromatography (TLC).

The sterile influent media for the chemostats consisted of the nitrate ester being tested, ethanol as an additional carbon source, and mineral salts. The starting esters and their nitrate-containing metabolites could be reduced to below detection levels in each case. Samples from the culture vessels and the effluent media revealed intermediate degradation products which had lower R_f values than the corresponding parent compounds and were positive for the presence of the nitrate group when the chromatograms were visualized with a nitrate spray reagent.

Tentative identification of the metabolites was established by cochromatography of the reaction products with standard metabolites whose syntheses will be described later. Each of the four propellant esters underwent biologically mediated denitration to give the corresponding mononitrates. Thus, PGDN gave propylene glycol mononitrate (PGMN), DEGDN gave diethylene glycol mononitrate (DEGMN), and TEGDN gave triethylene glycol mononitrate (TEGMN). The bicdegradation of TMETN also proceeded in steps, in this case giving two nitrogenous intermediates, trimethylolethane dinitrate (TMEDN) and trimethylolethane mononitrate (TMEMN).

[2] D. Kaplan, J. Walsh, and A. M. Kaplan. 1981. Decomposition of Glycols from Nitrate Ester Propellants. Technical Report NATICK/TR-81/017, US Army Natick Research and Development Laboratories, Natick, MA.

Further exposure of the reaction mixtures to the action of the microorganisms resulted in complete disappearance of all nitrate esters. Presumably, hydrolytic cleavage of the mononitrates resulted in the formation of the parent polyglycols, but this assumption was not confirmed experimentally. A subsequent investigation carried out in this laboratory demonstrated that the parent polyglycols undergo biodegradation under batch aerobic and anaerobic conditions.[3] They were also shown to possess at most a low order of toxicity. Transformations involved in the biodegradation of the nitrate esters are shown schematically in Figure 1. The identities of the intermediates were confirmed by comparison with

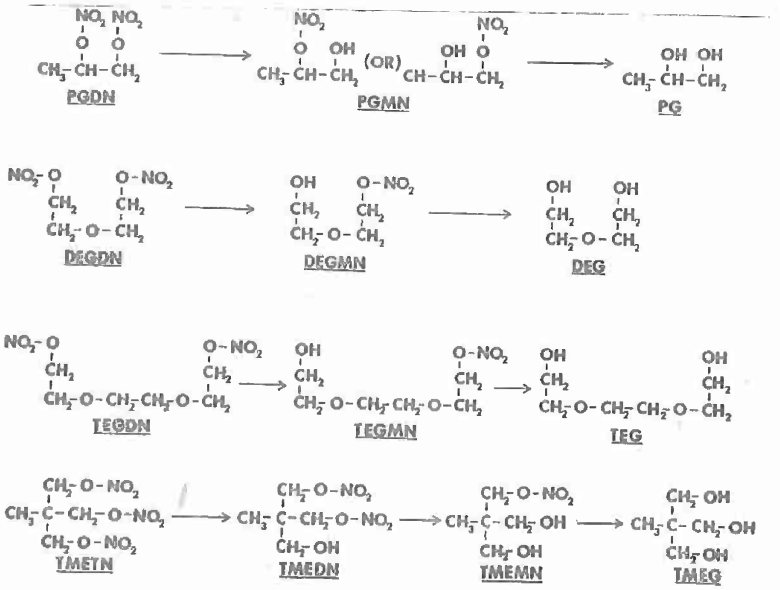

Figure 1. Biotransformation of the Nitrate Esters.

[3] See footnote 2, p. 6.

synthesized standards. The unknowns were extracted from the reaction mixtures with ether and identified by cochromatography (TLC) with the standards, and by GC/MS of the isolated fractions obtained by preparative TLC of the extracts (Figures 2 and 3).

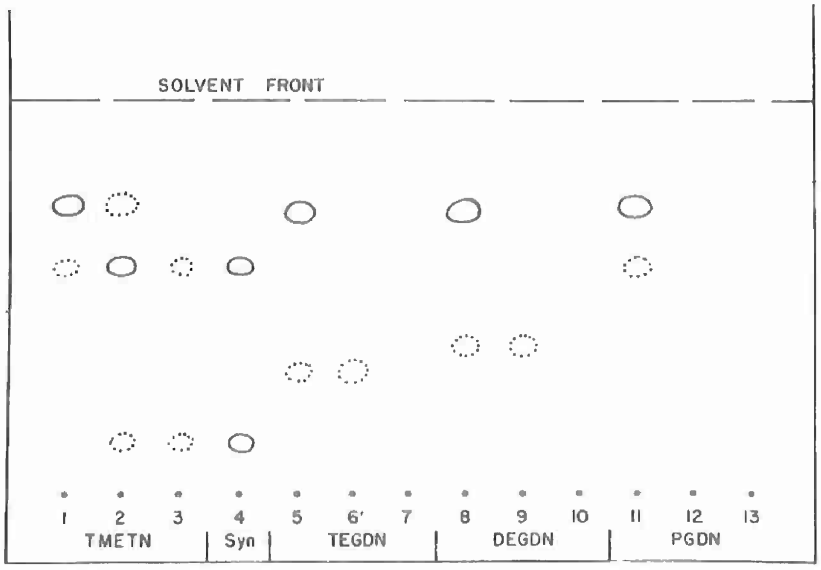

Figure 2. TLC of Batch Culture Degradation of Nitrate Esters. No. 4 is a Reference Sample Containing Synthetic TMEDN and TMEMN. Other Nos. Refer to Samples Taken from Shake Flasks at 48-hour Intervals.

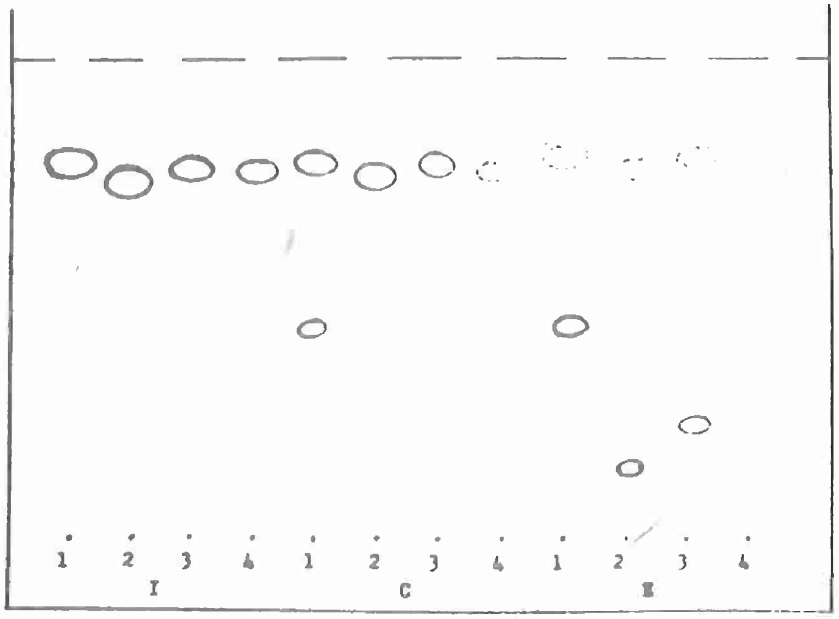

Figure 3. TLC of Continuous Culture Degradation of Nitrate Esters. Nos. 1-4 Refer to Chemostats Containing TEGDN, DEGDN, TMETN, and PGDN Respectively. I Refers to Influent, C to Chemostat Contents and E to Effluent.

The compounds required for identification of the intermediates isolated from culture extracts were synthesized by use of an acetate blocking group. The reaction scheme for trimethylolethane (TME) is shown in Figure 4. An excess of TME was treated with acetic anhydride; under these conditions the

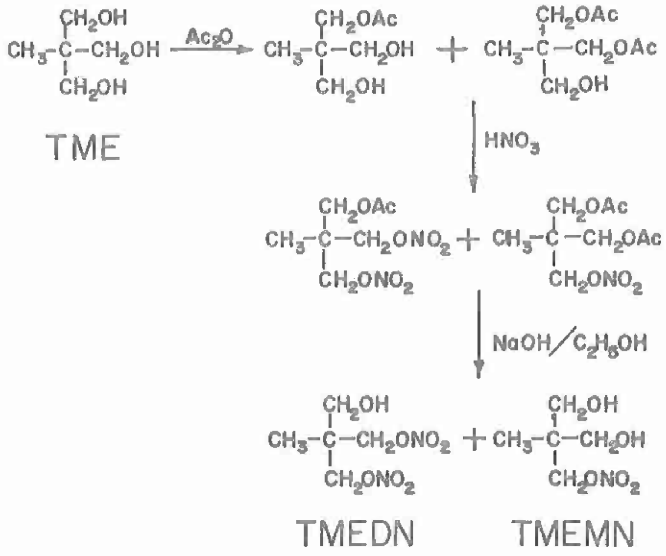

Figure 4. Scheme for Synthesis of TMEDN and TMEMN.

diacetate and monoacetate were the principal products. This mixture of acetates was treated with nitric acid which reacted with the remaining free hydroxy groups to give the corresponding nitrate esters. The acetate groups were then removed by selective saponification. The nitrate groups were left intact, thus yielding the dihydroxy mononitrate and the monohydroxy dinitrate.

The resulting mixture was separated by chromatography on silica gel. The properties of the isolated products agreed with the literature; their Infrared (IR) and MS conformed to the assigned structures.

The scheme for syntheses of the mononitrates of the remaining three nitrate esters is presented in Figure 5.

The original method attempted was that of direct nitration (Figure 5). We were successful in producing trace amounts of the glycol mononitrates using an excess of the glycol in question, but the yields were insufficient. Therefore, the acetate blocking group was again employed as for TME (Figure 5).

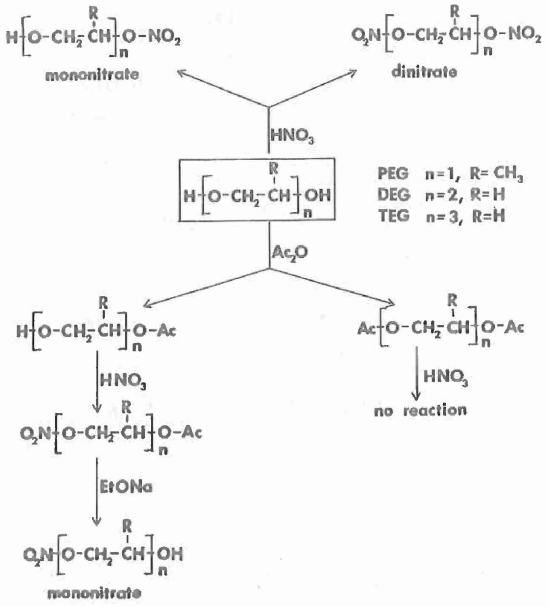

Figure 5. Scheme for Synthesis of Glycol Mononitrates.

This procedure resulted in a mixture of mono- and diacetates which was treated with concentrated nitric acid. The diacetate did not react under these conditions, but the main product was the mononitro-monoacetate. The acetate group was then selectively cleaved as before without affecting the nitro group. The main product, the glycol mononitrate, was obtained by chromatographic separation of the reaction mixture.

In the case of PGMN tne structure was ambiguous since the mononitrate could form with the nitrate group at the 1 or 2 position. The synthesis could, in principle, yield both isomers of 1,2-propanediol mononitrate. Attempts to separate the products by TLC yielded only one spot. That spot, however, did correspond to the spot obtained from the biological degradation of 1,2-propanediol dinitrate. Either the biological degradation and the synthetic methods gave the same isomer, or the isomers were not separated by TLC.

METHODS

Materials Propylene glycol, diethylene glycol, and triethylene glycol were purchased from the Fisher Scientific Company, Fair Lawn, NJ; trimethylolethane was purchased from Aldrich Chemical Company, Milwaukee, WI. One hundred percent nitric acid was prepared from fuming nitric acid, sp. g. 1.5 (obtained from Merck and Co., Rahway, NJ), by distillation from concentrated sulfuric acid.[4]

Propylene glycol dinitrate (PGDN), DEGDN, TEGDN, and TMETN were obtained from the Radford Army Ammunition Plant, Radford, VA.

Batch shake-flask experiments were carried out in 100-mL aliquots of medium contained in 250-mL Erlenmeyer flasks incubated at 30°C with shaking at 225 rpm in a New Brunswick G24 Environmental Incubator Shaker. Continuous culture studies were performed in 350-mL chemostats (New Brunswick, Bioflow Model C30) at 30°C; aeration, 0.8 Liter/min; detention time 8-15 hours.

TLC was performed with silica gel media (Eastman Chromagram 13179 without fluorescent indicator).

The chromatograms were developed with benzene-ethanol (95/5, v/v) in a Gelman Model 51325-1 saturation chamber. The nitrate esters were visualized by spraying with 5% diphenylamine in ethanol followed by exposure to an Ultraviolet (UV) lamp (Sylvania G15T8, germicidal) for 5-10 minutes. The nitrate esters produced brown to blue-gray colorations depending on their concentration.

[4] E. C. Horning. 1955. Tetranitromethane, Organic Syntheses, Coll. Vol. III, John Wiley and Sons. New York, NY. p. 802.

Preparative TLC was carried out with Whatman Type LK5DF 5 x 20-cm channelled plates with 250 μm silica gel layers and preabsorbent strips. The preabsorbent strips were moistened with the concentrated ethereal extracts from the nitrate ester biodegradation broths. The chromatograms were developed and visualized as before. The zones were located by masking most of the plate and spraying only a narrow vertical strip on either side. Zones representing each individual component were scraped off, combined with corresponding zones and extracted with 10% ethanol in acetone. The filtered extracts were evaporated to dryness and analyzed by GC/MS. Six plates were used for the analysis of each of the four nitrate esters.

Column chromatography was performed with silica gel, Davison Grade 62, Mesh 60-200, Code 62-08-08-226, purchased from the Davison Chemical Co., Baltimore, MD. IR spectra were obtained with a Perkin-Elmer Model 283 IR spectrophotometer. Melting points were taken with a Fisher-Johns melting point apparatus.

MS were obtained with a Finnigan Model 4000 quadrupole GC/MS equipped with an automatic data processing accessory. The mass spectrometer was operated in the chemical ionization mode with methane as reactive gas (15 mL/min). GC was carried out with a 6-foot (1.8 m) glass column packed with 3% SE30 on Supelcoport 80-100 Mesh with nitrogen as carrier. The injection port was maintained at 200°C. After injection the column oven was programmed to remain at 100°C for one minute, then to rise to 200°C at 10°/minute, and finally to remain at 200°C for 5 minutes.

Media. The basal medium for chemostat continuous culture experiments consisted of 11 mg of K_2HPO_4, 0.7 mg of NaCl, 3.6 mg of $MgSO_4 \cdot 7H_2O$, 0.57 mL of

ethanol, 0.07 mL of anhydrous ether and 2.86 mg of $NH_4H_2PO_4$ per Liter of distilled water at pH 6.8. The nitrate ester was added to the basal medium at a concentration of 30 mg/L.

The basal medium for batch shake-flask experiments consisted of 0.2 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of KH_2PO_4, 1.0 g of K_2HPO_4, 0.02 g of $CaCl_2$, and 0.05 g of $FeCl_3$ in 1 L of distilled water adjusted to pH 7.0. Glucose, 1.0 g/L and/or nitrate ester, 70 mg/L were added.

Inocula Inocula for the batch shake-flask cultures were obtained by inoculating nutrient broth with fresh activated sludge from a local domestic sewage treatment plant. The inocula were incubated in nutrient broth and then collected and washed by centrifugation prior to use. Inoculation of the continuous cultures was carried out by adding 10 mL of this aerated activated sludge to the chemostat culture vessel.

Assay of Nitrate Esters Microbial growth media were clarified by centrifugation and filtration. Aliquots of media were extracted with ethyl ether for 24 hours in a continuous liquid/liquid extractor. The extracts were concentrated at room temperature under a stream of nitrogen. Ten to 30 µL of the concentrate were spotted on silica gel TLC media which were developed and visualized as described before.

Preparation of OEGMN A 250-mL 3-neck flask was equipped with a condenser, thermometer, addition funnel, heating mantle and stirrer. Ninety mL of diethylene glycol were introduced into the flask and the temperature brought to 115°C with stirring. Heating was terminated and 60 mL of acetic anhydride were added at such a rate that the temperature did not exceed 135°C. The addition

required about 30 minutes. The reaction mixture was maintained at 115-120°C for 2 hours by further heating.

The reaction mixture was poured into an equal volume of water which was then neutralized with sodium bicarbonate. The precipitate of sodium acetate was removed by filtration and the filtrate was saturated with sodium chloride. It was extracted with three 150-mL portions of ethyl ether which were combined and dried over anhydrous calcium sulfate. The ether was removed on a rotary evaporator at room temperature. The yield of mixed diethylene glycol esters was 49.7 g.

A 10-g aliquot of the mixed diethylene glycol esters was added incrementally to 40 mL of 100% nitric acid over a period of 10 minutes. The reaction temperature was not allowed to exceed 15°C by means of an ice bath. The mixture was retained at ice bath temperature for an additional 5 hours. It was poured into 150 mL of water and an equal volume of ice. The solution was saturated with sodium chloride and extracted three times with 100-mL portions of ether. The combined ethereal extracts were neutralized with 10% sodium bicarbonate solution and dried over anhydrous calcium sulfate. The filtered extract was evaporated to an oily concentrate on a rotary evaporator at room temperature.

A 5-g aliquot of the concentrate, which consisted of mixed nitrate/acetate esters, was added at 10°C to 30 mL of a solution consisting of 5 g of sodium hydroxide, 10 mL of water and 30 mL of 95% ethanol. The reaction mixture was brought to 25°C and maintained at that temperature for 0.5 hours. It was then cooled in an ice bath and neutralized with 6N hydrochloric acid. The reaction mixture was then saturated with sodium chloride and extracted three times with an equal volume of ether. The extracts were dried over anhydrous calcium

sulfate, filtered, and the ether removed on a rotary evaporator. The yield was 3.75 g of an oily residue consisting of a mixture of DEGMN and DEGDN.

Chromatography of DEGMN and DEGDN A 22-mm ID column was packed with a slurry of silica gel in dichloromethane to give a 17-cm packed section. One g of the DEGMN/DEGDN mixture was spiked with about D.25 mg each of aminoanthroquinone and picric acid to act as dye markers and applied to the column. The column was successively eluted with dichloromethane, ether, and ethanol while 20-mL fractions were collected. Fractions eluting between the dye markers were assayed by TLC and those that were essentially pure DEGMN were combined and the solvents removed by rotary evaporation. The yield of DEGMN was D.27 g of an oily liquid.

The IR spectrum of DEGMN (Figure 6) conforms to the structure of the compound, exhibiting bands at 345D cm^{-1} (- OH) and at 1640 cm^{-1} (- ONO_2). It agreed with the spectrum reported by Rossmy for this compound.[5] The structure was also confirmed by the presence of a (M + 1) ion at m/z 152 in the MS.

Preparation of TEGMN The preparation and isolation of TEGMN was carried out in the same manner as DEGMN. The product was a viscous oily liquid whose IR spectrum (Figure 7) was consistent with the structure. The identity of the product was confirmed by the presence of an (M + 1) ion at m/z 196 in the MS.

[5] G. Rossmy. 1955. Reaction of Ethylene oxide with Dinitrogen Tetroxide. Chem. Ber. 88: 1969-72.

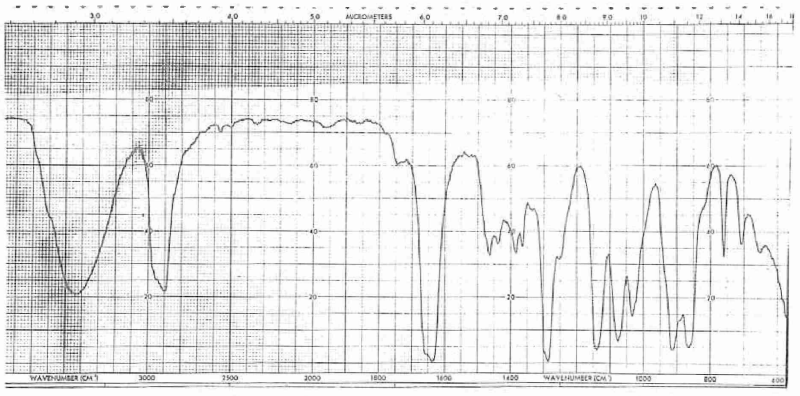

Figure 6. IR Spectrum of DEGMN.

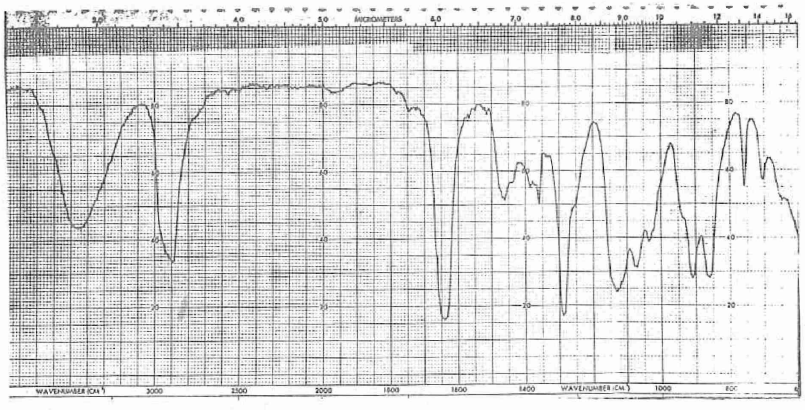

Figure 7. IR Spectrum of TEGMN.

Preparation of PGMN There are two possible isomers of PGMN, 1-nitroxy-2-propanol and 2-nitroxy-1-propanol. Pujo and Boileau have reported the preparation of PGMN of unknown isomeric composition by reaction of propylene oxide with nitrogen tetroxide.[6,7] Litchfield reoorted the preparation of both PGMN isomers by reaction of the corresponding bromohydrins with silver nitrate; however no details were given.[8]

In the present investigation PGDN was synthesized in the same way as DEGDN. The product was an oily liquid whose IR spectrum and MS conformed to the structure. It is not known which of the two PEDGN isomers was obtained, or whether the product was a mixture of isomers. The TLC gave no evidence of the presence of more than one compound; however, the method may have lacked sufficient resolution to separate these isomers if both had been present.

Preparation of TMEDN and TMEMN Partially nitrated derivatives of trimethylolethane were prepared by a modification of the method of Marans and Preckel.[9] Three g of trimethylolethane were treated with 3 mL of acetic anhydride for 1.25 hours at 115-120°C. The reaction mixture was poured on an excess of 5% sodium bicarbonate solution which was then extracted with ethyl ether in a continuous liquid-liquid extraction apparatus for 18 hours. The extracts were dried over anhydrous calcium sulfate, filtered, and the ether removed by rotary evaporation.

[6] A. Pujo, and J. Boileau. 1955. Action of Nitrogen Peroxides on Epoxides, Mem. Poudres. 37: 35-48.

[7] A. Pujo, and J. Boileau. 1953. New Synthesis of Nitrite Nitrates, Compt. Rend. 237: 1422-4.

[8] M. H. Litchfield. 1968. The Determination of the Di- and Mononitrates of Ethylene Glycol and 1,2-Propylene Glycol. Analyst (London) 93: 653-9.

[9] N. S. Marans, and R. F. Preckel. 1954. Nitrate Esters of 2,2-Dimethylol 1-propanol Monoacetate and Diacetate. J. Am. Chem. Soc. 76: 3223.

The residue was added incrementally to 12 mL of 100% nitric acid which was concurrently stirred in an ice bath. The reaction was stirred for an additional 2 hours in the ice bath and was then poured onto an ice and water mixture. The product was extracted with dichloromethane, washed with 5% sodium bicarbonate, water, and dried over anhydrous calcium sulfate.

A solution of alcoholic sodium hydroxide was prepared consisting of 5 g of sodium hydroxide, 10 mL of water, and 30 mL of 95% ethanol. The residue from the evaporation of the dichloromethane was treated with 15 mL of the alcoholic sodium hydroxide at 23-25^{o}C for 25 minutes. The reaction mixture was neutralized with dilute hydrochloric acid, saturated with sodium chloride, and extracted two times with an equal volume of ether. The combined ethereal extracts were dried over anhydrous calcium sulfate, filtered, and the solvent evaporated. The residue deposited several crops of crystals of TMEMN which were collected and recrystallized from benzene, mp 74.5-75.5^{o}C; lit. mp 76-77^{o}C. The IR spectrum of the product (Figure 8) conformed to the structure of TMEMN exhibiting bands for -OH and -ONO_2. The structure was further substantiated by the presence of a peak in the MS at m/z 166 corresponding to the (M + 1) ion.

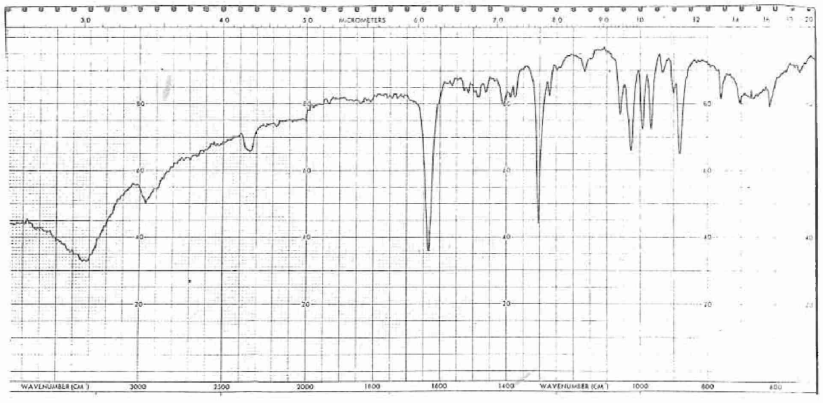

Figure 8. IR Spectrum of TMEMN.

Chromatography of TMEDN and TMEMN The mother liquor from the crystallization of TMEMN was evaporated until most of the solvent had been removed. It was chromatographed on a 20-mm ID column, slurry-packed with benzene-silica gel to give a 20-cm packed section.

The column was eluted successively with 50 mL each of benzene, benzene/ethanol (97.5/2.5), benzene/ethanol (95/5), benzene/ethanol (90/10), and finally with 100 mL of acetone. Arbitrary 10-mL fractions were taken and subsequently assayed for the nitrate esters by TLC. Fractions containing essentially pure TMEDN were combined and the solvent removed by rotary evaporation. The product was an oily liquid whose IR spectrum (Figure 9) conformed to the structure of TMEDN. The presence of a peak in the MS at m/z 211 corresponded to the (M + 1) ion of TMEDN.

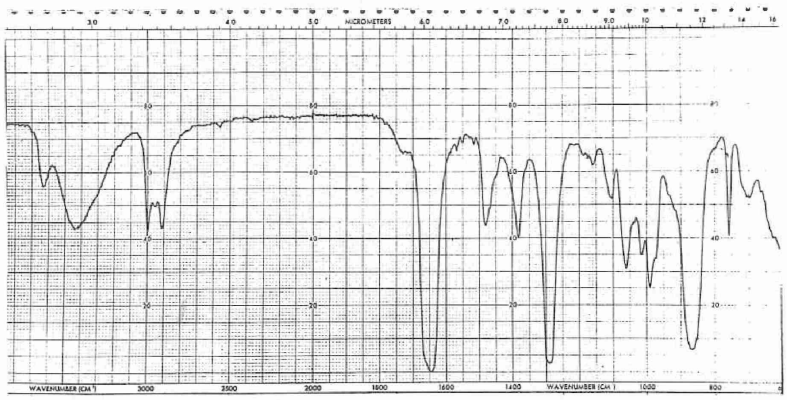

Figure 9. IR Spectrum of TMEDN.

CONCLUSIONS

The four propellant esters, PGDN, DEGDN, TEGDN, and TMETN undergo biotransformation by microorganisms in either batch or continuous cultures under aerobic conditions. The degradation proceeds via a sequential stepwise hydrolytic cleavage of the nitrate groups resulting in the formation of the partially nitrated parent glycols. These intermediates were also prepared by chemical synthesis to provide standards for identification of the biologically mediated intermediates.

The disappearance of the mononitrate esters suggests that they were converted to the corresponding glycols. This hypothesis could not be verified experimentally at the time the studies were completed because definitive analytical procedures for the glycols did not then exist. Subsequently, methods for the analysis of trace amounts of the glycols in aqueous media were developed which lead to the conclusion that the parent glycols did undergo degradation by microorganisms and other environmental agents.[10]

[10] See footnote 2, p. 6.

RECOMMENDATION

The present work suggests that waste streams containing the four esters singly or in combination with nitroglycerinc could be detoxified by the same microbial process suitable for nitroglycerine.

Further development of this process should involve confirmation that the parent glycols are formed and determination of the kinetics of the various biotransformations. Additional work on the toxicity and mutagenicity of the nitrate intermediates would be advisable, as well as further biodegradation studies involving mixtures of the esters and nitroglycerine.

REFERENCES CITED

Horning, E. C. 1955. Tetranitromethane, Organic Syntheses. Coll. Vol. III. John Wiley and Sons. New York, NY. p. 802.

Kaplan, D., J. Walsh, and A. M. Kaplan. 1981. Decomposition of Glycols from Nitrate Ester Propellants. Technical Report NATICK/TR-81/017, US Army Natick Research and Development Laboratories, Natick, MA.

Litchfield, M. H. 1968. The Determination of the Di- and Mononitrates of Ethylene Glycol and 1,2-Propylene Glycol. Analyst (London) 93: 653-9.

Marans, N. S. and R. F. Preckel. 1954. Nitrate Esters of 2,2-Dimethylol 1-Propanol Monoacetate and Diacetate. J. Am. Chem. Soc. 76: 3223.

Pujo, A., and J. Boileau. 1955. Action of Nitrogen Peroxides on Epoxides. Mem. Poudres. 37: 35-48.

Pujo, A., and J. Boileau. 1953. New Synthesis of Nitrite Nitrates. Compt. Rend. 237: 1422-4.

Rossmy, G. 1955. Reaction of Ethylene Oxide with Dinitrogen Tetroxide. Chem. Ber. 88: 1969-72.

Wendt, T. M., J. H. Cornell, and A. M. Kaplan. 1978. Microbial Degradation of Glycerol Nitrates. Appl. Env. Microbiol. 36: 693-699.